Low vitamin B6, and not plasma homocysteine concentration, as risk factor for abdominal aortic aneurysm: A retrospective case–control study

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Background: **Hyperhomocysteinemia has been associated with vascular disease in many epidemiologic studies, but only a few have reported on the relation between hyperhomocysteinemia and aneurysms of the abdominal aorta (AAAs). Although these studies showed higher homocysteine concentrations in patients with AAA than in controls, little attention had been given to possible confounding factors. Most patients with AAA are of older age, have an impaired renal function, and have other risk factors for cardiovascular disease. This matched case–control study investigated the relation between homocysteine concentration (before and after methionine loading) and AAA, taking into account possible confounders such as age, sex, and concentrations of creatinine and B vitamins.**

Methods: **Patients with a history of AAA were recruited from the outpatient clinic; 60% had already undergone surgery for their AAA. They were asked to invite a friend or neighbor to participate as a control subject (age-matched and sex-matched). Concentrations of homocysteine, vitamin B6, vitamin B12, folate, and creatinine were determined in the fasting state, and blood was taken for methylenetetrahydrofolate reductase (MTHFR) mutation analysis. Six hours after oral methionine loading, the postmethionine load homocysteine concentration was determined.**

Results: **Univariate analysis showed an odds ratio (OR) of 2.2 (95% confidence interval (CI), 0.9 to 5.5) for the risk of AAA for the highest quartile of homocysteine concentration. After adjustment for creatinine, the OR was markedly reduced to 1.24 (95% CI, 0.42 to 3.66), and this risk further attenuated in the multivariate analysis. Univariate analysis of the B vitamins showed an increased risk of AAA for the bottom quartile of vitamin B6 (OR, 3.75; 95% CI, 1.22 to 11.54), which even increased after adjustments. The relative risk associated with the MTHFR 677TT polymorphism was 2.1 (95% CI, 0.9 to 5.3).**

Conclusion: **Vitamin B6, but not homocysteine, is an independent risk factor for AAA. The role of vitamin B6 in the pathogenesis of AAA needs to be further elucidated. (J Vasc Surg 2007;45:701-5.)**

Abdominal aortic aneurysm (AAA) is present in 4.3% to 8.8% of men aged >65 years, and rupture of AAA causes 1% to 2% of all deaths in men aged >65 years in Western countries.[1] The incidence of AAA is increasing, partly owing to ageing of the population. The insights on the pathogenesis of AAA have been changed in recent decades. In the past, AAA was regarded as a consequence of atherosclerosis.[1,2] More recent studies suggest a significant role for matrix metalloproteinases (MMPs) in the pathogenesis of AAA.[3,4] MMPs regulate the turnover of extracellular matrix proteins. Tissue inhibitors of MMPs (TIMPs) inhibit active enzymes and are also present in increased amounts in the wall of the aneurysm. So the net effect in tissues depends on the balance between MMPs and TIMPs. In patients with AAA, this balance shifts towards proteolytic activity.[3,4]

Hyperhomocysteinemia is thought to be a risk factor for arterial vascular disease[5] and venous thrombosis.[6] Studies on the methylenetetrahydrofolate reductase (MTHFR) 677TT genotype suggested that this relationship was causal,[7] but two recently published trials did not show an effect of homocysteine-lowering with B vitamins on cardiovascular end points.[8,9] Only a few studies have been published on the relation of homocysteine and AAA.[10-13] Although these studies showed that patients with AAA have higher plasma concentrations of homocysteine, they did not take into account that homocysteine concentration is dependent on several other factors such as age, sex, B vitamins, and renal function.

We evaluated the role of homocysteine in AAA, taking into account possible confounders by initiating a case–control study in patients with AAA and control subjects of the general population. In addition, we studied determinants of homocysteine concentration, such as B vitamins and creatinine, performed a methionine-loading test, and determined the MTHFR genotype.

METHODS

Study population. The study protocol has been described before.[14] Briefly, all patients with AAA who visited

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the outpatient clinic of the TweeSteden Hospital, Tilburg, a regional hospital in a city of 200,000 inhabitants in The Netherlands, were invited to take part in this study. We invited 149 patients who had already undergone surgery for their AAA as well as patients who had not had surgery for their AAA (defined as ultrasonographically proven infrarenal aortic diameter ≥30 mm). Of these, 89 patients wanted to participate (response rate, 60%), of whom 53 (60%) had already undergone surgery.

The concentrations of homocysteine, B vitamins, and folate are determined mostly by the factors of age, diet, smoking, and for homocysteine, especially, renal function. We do not think that these are influenced by surgery for AAA. We therefore grouped the patients who underwent surgery and the patients who did not have surgery for their AAA.

Each patient was asked to invite a friend or neighbor of the same sex and within a 5-year age range to serve as a control. We failed to match four patients. For three, we included a family member as a control that was matched to another patient. We could not match one 94-year-old woman, and she was excluded. Our final study population consisted of 88 patients with AAA and 88 control subjects matched for age and sex.

All control subjects underwent ultrasonography of the abdominal aorta. Control subjects with an aortic diameter ≥30 mm were excluded. All subjects were asked to complete a questionnaire about medical and family history, medication use (including vitamins), and smoking habits. In The Netherlands cereal grains are not supplemented with folate. Blood pressure was measured in the supine position, three times with a mercury sphygmomanometer, after a resting period of 10 minutes. All participants gave written informed consent, and the TweeSteden Hospital Medical Ethics Committee approved the study protocol.

Determinants of homocysteine concentration, methionine loading test, and MTHFR genotype. After an overnight fast, blood was taken for determination of plasma concentration of homocysteine, vitamin B6 (pyridoxal 5-phosphate), B12 (cobalamin), folate, and creatinine, as well as MTHFR mutation analysis. Then subjects underwent a methionine-loading test. Six hours after an oral loading dose of L-methionine (0.1 g/kg body weight in 200 mL orange juice),[15] blood was taken to determine a postmethionine load homocysteine concentration.

Total homocysteine concentrations were measured according to the method described by Araki and Sako.[16] Folate and cobalamin concentrations were measured with the competitive protein-binding assay on the Technicon Immuno 1 System (Technicon Instruments, Tarrytown, NY). Determination of pyridoxal 5-phosphate was performed by high performance liquid chromatography according to Ubbink et al.[17] DNA was isolated from the buffy coat, and mutation analysis was accomplished by means of polymerase chain reaction as described elsewhere.[18,19] The primers generate a 198-bp fragment. The MTHFR C→T substitution at bp 677 creates a Hin*fI* recognition sequence. If the mutation is present, Hin*fI* digests the 198-bp fragment into a 175-bp and a 23-bp fragment. The fragments were analyzed by polyacrylamide gel electrophoresis.

Table I. Characteristics of patients with abdominal aortic aneurysm and control subjects

Characteristic	*Patients (n = 88)*	*Controls (n = 88)*	P
Age in years (range)	69 (45-85)	67 (44-83)	NS
Male/female	81:7	81:7	NS
Aneurysm			
Not operated	35		
Operated	53		
Family history of aneurysm	16	9	NS
History			
Myocardial infarction	26	9	<.01
Cerebrovascular disease	9	4	NS
Pulmonary embolism	2	3	NS
COPD	24	13	.04
Peripheral vascular disease	19	4	<.01
Cigarette smoking (pack-year ± SD)*	34 ± 33	23 ± 22	<.01
Mean blood pressure			
Systolic, mm Hg ± SEM	159 ± 2.4	158 ± 2.7	NS
Diastolic, mm Hg ± SEM	85 ± 1.2	87 ± 1.2	NS
Creatinine (μmol/L)	101.4	90.5	<.01
ACE inhibitors	19	10	.07
β-blockers	25	13	.03
Statins	22	6	<.01
Vitamin status			
Use of B vitamins	17	16	NS
Vitamin B6 (nmol/L)	67.2	75.7	.08
Vitamin B12 (pmol/L)	229.5	263.5	.04
Folate (nmol/L)	13.1	13.7	NS

NS, Not significant; *COPD,* chronic obstructive pulmonary disease; *SEM,* standard error of the mean; *ACE,* angiotensin-converting enzyme.
*A pack-year was defined as smoking 20 cigarettes/day for 1 year.

Statistical analysis. General baseline characteristics were compared using the Student unpaired two-tailed *t* test and χ^2 test. To investigate the relative risks, we calculated crude and adjusted matched odds ratios (OR) and 95% confidence intervals (CI) with a conditional logistic regression model using Stata 8 (StataCorp, College Station, Tex). Concentrations of homocysteine, vitamin B6, B12, and folate were divided into quartiles and analyzed as categoric variables to evaluate both the risk and dose-response relationship. We calculated the risk (crude ORs) to have an AAA for the different quartiles and used either the top or bottom quartile as the reference category. We used a multivariate model to adjust this OR first for creatinine, then for each other, and for smoking. Finally, we adjusted the ORs for the classic risk factors for cardiovascular disease such as hypertension and dyslipidemia.

RESULTS

The median age was 69 years (range, 45 to 85 years) for the patient group and 67 years (range, 44 to 83 years) for the control group. The male/female ratio was 81:7. An open surgical procedure was done in 53 (60%) of the 88 patients. Elective surgery was performed in 40 patients.

Table II. Crude and adjusted odds ratios for the risk of abdominal aortic aneurysm for quartiles of homocysteine, vitamin B6, B12, and folate concentrations

Variables (in quartiles)	*Crude OR (95% CI)*	*Adjusted OR* (95% CI)*	*Adjusted OR† (95% CI)*
Plasma homocysteine (μmol/L)			
<9.33	reference category	reference category	reference category
9.33-11.8	2.02 (0.87-4.68)	1.94 (0.78-4.80)	1.09 (0.31-3.84)
11.8-15.1	2.06 (0.79-5.34)	1.45 (0.49-4.26)	1.15 (0.26-5.10)
≥15.1	2.22 (0.90-5.49)	1.24 (0.42-3.66)	0.78 (0.17-3.62)
Plasma vitamin B6 (nmol/L)			
<55	3.75 (1.22-11.54)	6.92 (1.63-29.28)	19.43 (2.12-177.78)
55-66	5.11 (1.78-14.70)	7.64 (2.19-26.61)	17.66 (2.95-105.78)
66-79	3.40 (1.24-9.34)	4.18 (1.33-13.13)	5.61 (1.38-22.82)
≥79	reference category	reference category	reference category
Plasma vitamin B12 (pmol/L)			
<171.5	3.16 (1.23-8.14)	3.26 (1.14-9.33)	0.95 (0.19-4.70)
171.5-224.5	2.54 (0.99-6.52)	2.53 (0.86-7.37)	1.52 (0.33-6.96)
224.5-301.5	1.16 (0.49-2.75)	1.02 (0.40-2.61)	0.42 (0.11-1.60)
≥301.5	reference category	reference category	reference category
Plasma folate (nmol/L)			
<8.73	2.13 (0.86-5.24)	1.76 (0.65-4.77)	0.51 (0.10-2.65)
8.73-11.6	1.55 (0.69-3.50)	1.23 (0.50-3.02)	0.46 (0.11-1.92)
11.6-15.5	1.75 (0.78-3.93)	1.41 (0.59-3.39)	0.99 (0.32-3.08)
≥15.5	reference category	reference category	reference category

OR, Odds ratio; *CI*, confidence interval.
*Adjusted for creatinine concentration.
†Adjusted for creatinine concentration, for each other, and smoking.

The AAA was symptomatic in seven and ruptured in six. Median time after surgery was 36 months (range, 4 to 132 months). The other 35 patients were followed up with ultrasonography. Other general characteristics of the patient and control group are summarized in Table I.

In the univariate analysis (Table II), we found an OR of 2.2 (95% CI, 0.9 to 5.5) for the risk of AAA for the highest quartile of homocysteine concentration. After adjustment for creatinine concentration, this risk estimate was markedly reduced to an OR of 1.24 (95% CI, 0.42 to 3.66), and after adjustments for B vitamins and smoking, the risk for AAA with a high homocysteine concentration was further attenuated. Univariate analysis of the B vitamins, as determinants of the homocysteine concentration, showed an increased risk for AAA for the bottom quartile of vitamin B6, B12, and folate (Table II). After adjustment for creatinine concentration, the risk for AAA was even more pronounced for the bottom quartile of B6 (OR, 6.92; 95% CI, 1.63 to 29.28), which remained after adjustments for smoking and concentrations of homocysteine, vitamin B12, and folate.

Although it is not evident whether hypertension and hypercholesterolemia are risk factors for AAA, we additionally corrected the OR for both factors, which did not change the high relative risk on AAA (data not shown).

Subgroup analysis of patients who underwent surgery and those who did not showed comparable results. The vitamin B6 concentration and distribution over the quartiles is presented for cases and controls in the Fig.

Univariate analysis of the postmethionine load homocysteine concentration showed no evident risk for AAA for the postload homocysteine concentration (Table III). After correction for creatinine concentration, the ORs also attenuated; after adjustments for B vitamins, no relation was found between the postload homocysteine concentration and the risk for AAA.

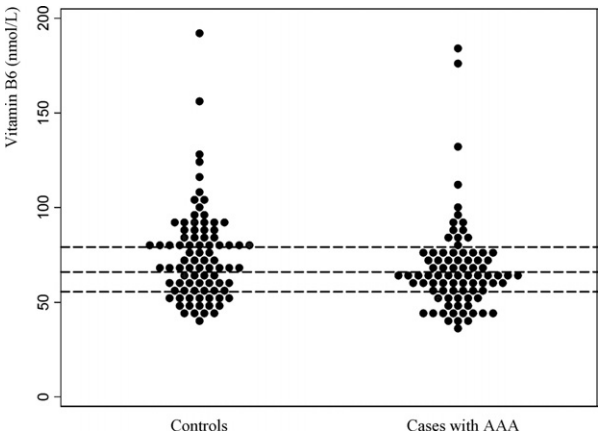

Fig. Dot plot of vitamin B6 concentration and distribution in quartiles, for controls and cases with abdominal aortic aneurysm.

Finally, we evaluated the 677 C→T mutation in the MTHFR gene, which is a common cause of hyperhomocysteinemia. Of the 85 patients, 15 were homozygous for the 677 C→T mutation compared with seven of the 86 control subjects. We calculated a matched OR of 2.1 (95% CI, 0.9 to 5.3).

DISCUSSION

Although homocysteine concentration seems to be a risk factor for AAA in univariate analysis, several indications

Table III. Crude and adjusted odds ratios for the risk of abdominal aortic aneurysm for quartiles of postmethionine load homocysteine concentration

Quartiles (μmol/L)	*Crude OR (95% CI)*	*Adjusted OR* (95% CI)*	*Adjusted OR† (95% CI)*
<27.7	reference category	reference category	reference category
27.7-33.4	0.99 (0.40-2.49)	0.98 (0.37-2.57)	0.63 (0.15-2.66)
33.4-40.9	2.95 (1.24-6.98)	2.39 (0.97-5.91)	1.33 (0.39-4.48)
≥40.9	1.48 (0.63-3.46)	0.95 (0.37-2.48)	1.05 (0.26-4.22)

OR, Odds ratio; *CI,* confidence interval.
*Adjusted for creatinine concentration.
†Adjusted for the concentration of creatinine, vitamin B6, B12, and folate, and for smoking.

show that this risk association is confounded by other factors, especially renal function. Homocysteine is strongly increased in renal dysfunction, and many patients with AAA have impaired renal function. That homocysteine after methionine loading (which is less influenced by renal function) is not a risk factor for AAA supports the hypothesis that homocysteine is not causally related to AAA, because in other forms of cardiovascular disease, fasting and postmethionine load homocysteine concentrations are both risk factors.[20]

A few other studies on the relation between hyperhomocysteinemia and AAA have found an impressive risk for AAA, with ORs of 8 to 36,[10-13] but these studies did not take renal function into account. To determine whether a possible association of homocysteine with AAA could have a causal relationship without influence of confounding factors, we analyzed the MTHFR genotype according to the principles of Mendelian randomization.[21] Our study showed a risk estimate for AAA with the MTHFR 677TT genotype of 2.1 (95% CI, 0.9 to 5.3). Two other studies also investigated the relation of homocysteine and AAA, as well as the MTHFR-genotype and AAA in the same population.[10,13] They also saw only a modest, not significant, relation between the MTHFR 677TT genotype and the occurrence of AAA.

In our multivariate analysis, the high risk-estimate for AAA with a low concentration of vitamin B6 is striking and remained after adjustments for the other variables and smoking. Earlier, the risk of a low B vitamin concentration was attributed to a higher homocysteine concentration, but in our multivariate analysis the high risk of AAA with a low vitamin B6 was independent of the basal homocysteine concentration. The concentration of B vitamin is not dependent on age, sex, renal function, or cardiovascular comorbidity and seems therefore to be less influenced by possible confounding factors. Other studies have also reported a homocysteine-independent risk of low vitamin B6 for arterial vascular disease.[22,23]

How could a low vitamin B6 status affect the abdominal wall? Collagen and elastin are major components of the vascular wall. In aneurysms, the elastic lamellae of the vascular wall are degraded, and collagen production is enhanced. Pyridoxal 5-phosphate is an important cofactor for lysyl oxidase, an enzyme responsible for cross-linking collagen and elastin. Animal studies have reported that pyridoxal 5-phosphate depletion inhibits lysyl oxidase.[24,25] Homocysteine did not inhibit lysyl oxidase. Pyridoxine deficiency was also responsible for alterations in bone collagen[26] and for loss of connective tissue integrity.[27] Further studies are needed to elucidate the role of vitamin B6 in the risk of AAA.

In our study, 53 of 88 patients had already undergone surgery for their aneurysm. We do not think that surgery directly influences homocysteine or vitamin concentrations. However, patients with large aneurysms who undergo surgery do more often have a decrease in renal function that results in an increase in homocysteine (but not vitamin) concentration. The MTHFR genetic polymorphism is not influenced by surgery.

CONCLUSION

Fasting homocysteine concentration was associated with AAA, but after adjustment for creatinine and B vitamin status, this association disappeared. However, a low concentration of vitamin B6 remained a risk factor for the occurrence of AAA. The role of vitamin B6 in the pathogenesis of AAA needs to be further elucidated.

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